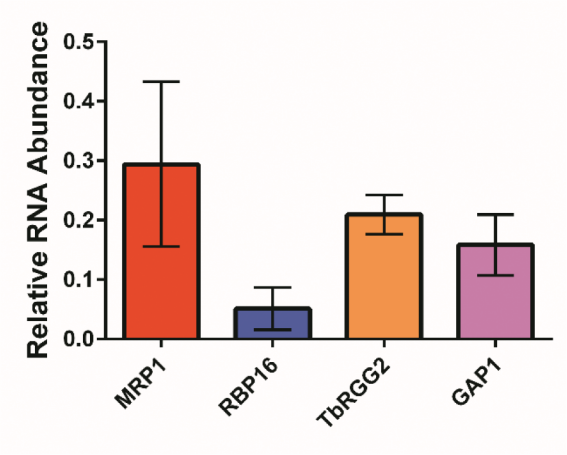
Figure S1

A

RNAi Cell Line	Rep1 - Tet	Rep1 + Tet	Rep2 - Tet	Rep2 + Tet
MRP1/2	1.2×10^7	9.2×10^6	7.4×10^6	5.2×10^6
RBP16	1.1×10^7	4.7×10^6	1.1×10^7	5.0×10^6
TbRGG2	4.6×10^6	3.0×10^6	8.0×10^6	9.4×10^6
GAP1	5.0×10^6	4.7×10^6	1.0×10^7	9.7×10^6

B



C

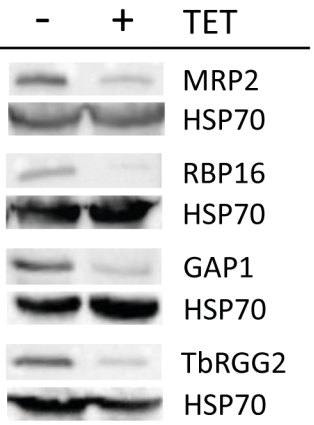


Figure S2

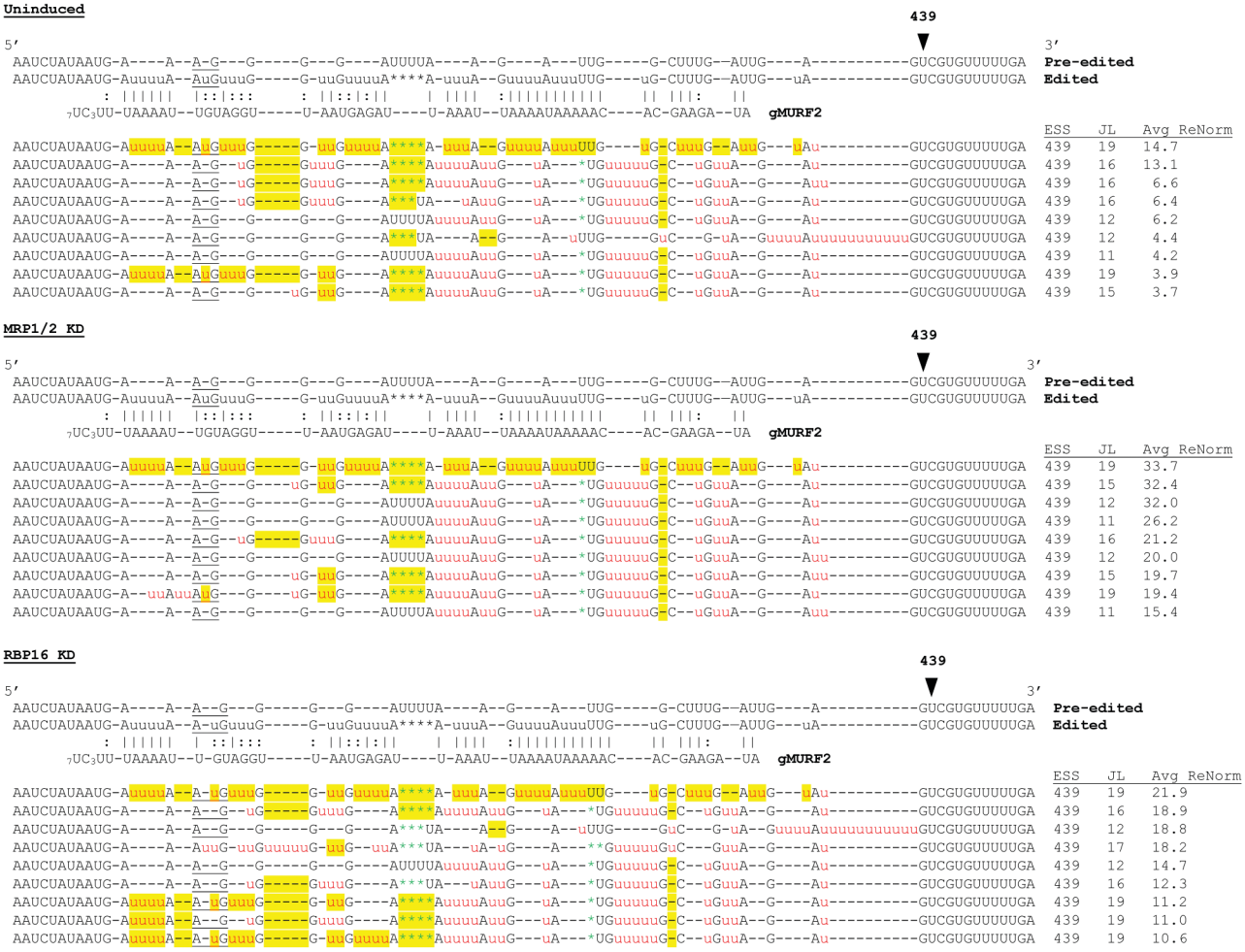
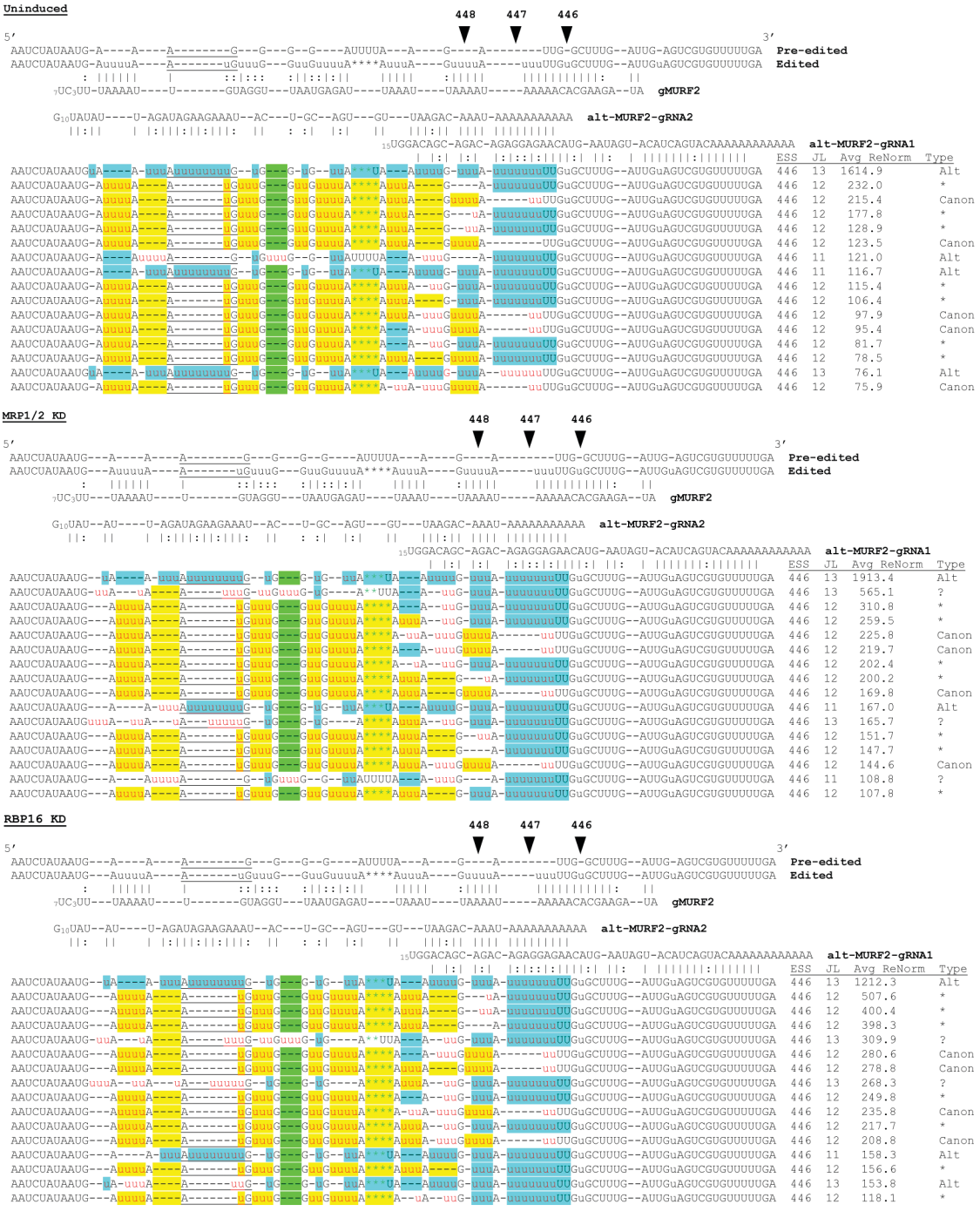

Uninduced
439
5′
3′
Pre-edited
Edited
gMURF2
ESS JL Avg ReNorm
MRP1/2 KD
RBP16 KD

Figure S3

A


Uninduced
MRP1/2 KD
RBP16 KD
448
447
446
Pre-edited
Edited
gMURF2
alt-MURF2-gRNA2
alt-MURF2-gRNA1
ESS JL Avg ReNorm Type

B

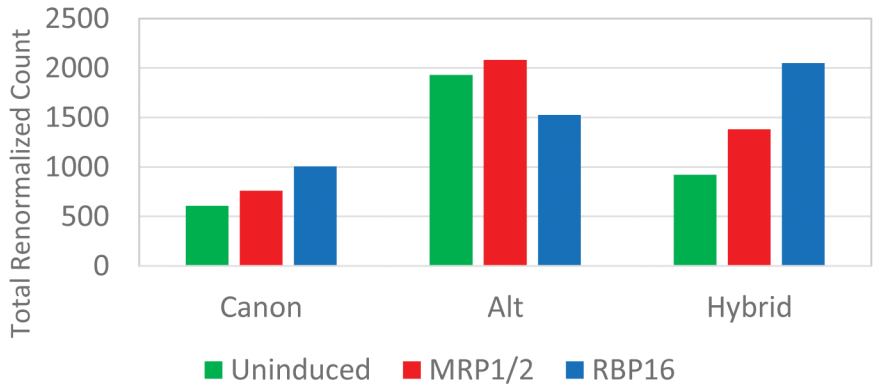

Total Renormalized Count
2500
2000
1500
1000
500
0
Canon
Alt
Hybrid
Uninduced
MRP1/2
RBP16

Figure S4

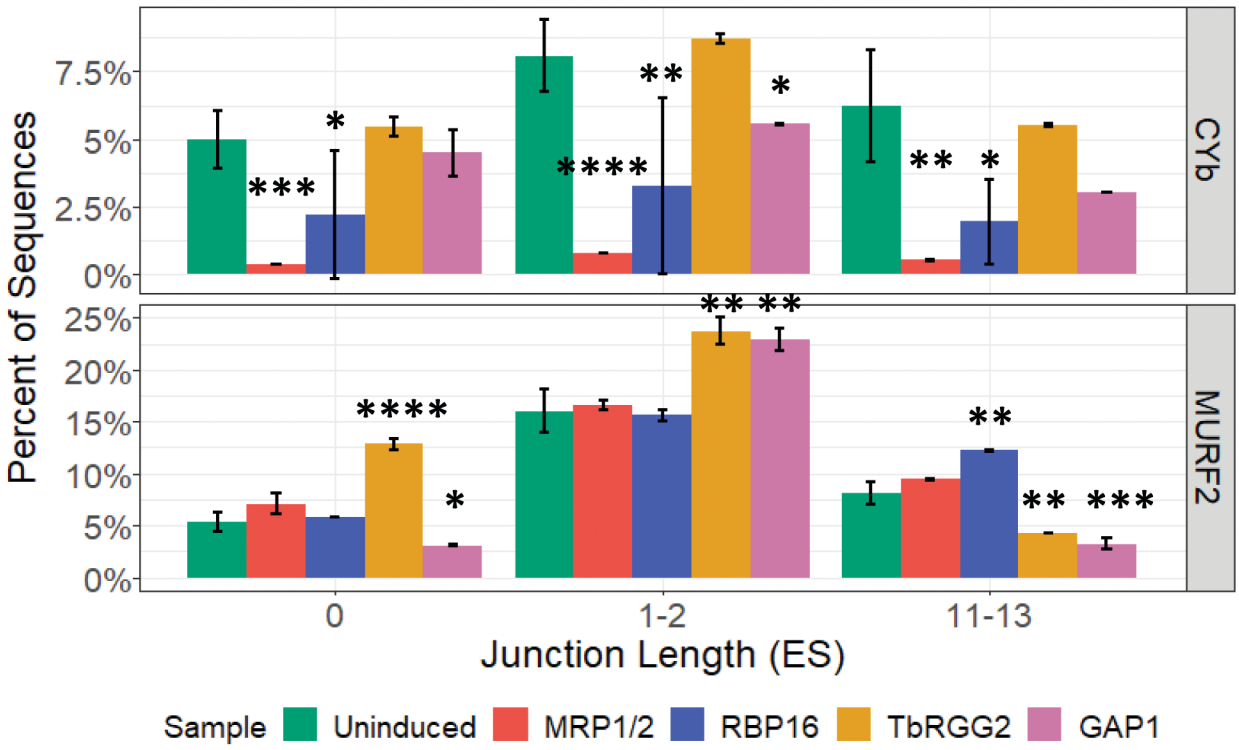

Percent of Sequences
7.5%
5%
2.5%
0%
25%
20%
15%
10%
5%
0%
CYb
MURF2
0
1-2
11-13
Junction Length (ES)
Sample
Uninduced
MRP1/2
RBP16
TbRGG2
GAP1

Figure S5

A


Uninduced
448
447
446
5′
3′
Pre-edited
Edited
gMURF2
alt-MURF2-gRNA2
alt-MURF2-gRNA1
TbRGG2 KD
GAP1 KD

B

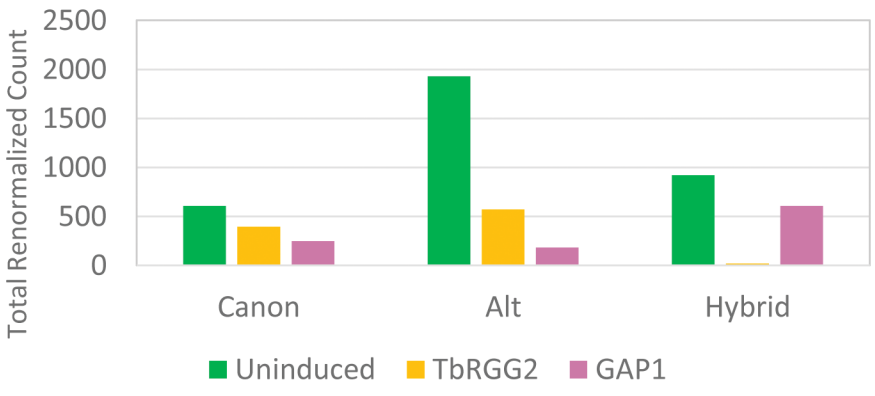

Total Renormalized Count
2500
2000
1500
1000
500
0
Canon
Alt
Hybrid
Uninduced
TbRGG2
GAP1

Fig. S1. RNAi cell lines analyzed in this study. (A) The concentration (cells/mL) of all uninduced (-Tet) and induced (+Tet) RNAi cell lines at time of RNA collection (day 3 post-induction). (B) Relative abundances (induced/uninduced) of target RNAs analyzed by qRT-PCR at the time of RNA collection (n=6 replicates). (C) Western blot analysis of target RNAs in uninduced (-Tet) and induced (+Tet) RNAi cell lines at time of RNA collection. HSP70 is a loading control.

Fig. S2. Sequence analysis of the most abundant junctions arising at MURF2 ESS439 in Uninduced, MRP1/2, and RBP16 cell lines. For each cell type the renormalized counts of junction sequences were averaged across all uninduced cell lines in this study (n=8) and induced replicates for each knockdown (n=2 for each cell line). The nine most abundant junctions arising in each cell type were then aligned according to their non-U sequence. MURF2 pre-edited and fully edited sequence, along with the previously identified gRNA-MURF2, are shown above. Editing sites that match the canonical sequence are highlighted in yellow. Lower case u's are those inserted by editing, U indicates encoded uridines, and * indicates encoded uridines deleted by editing.

Fig. S3. Sequence analysis of the most abundant junctions arising at MURF2 ESS446 in Uninduced, MRP1/2, and RBP16 cell lines. (A) For each cell type, the renormalized counts of junction sequences were averaged across all uninduced cell lines used in this study (n=8) and induced replicates (n=2 for each knockdown). To evaluate gRNA usage, the 16 most abundant junctions of lengths 11 editing sites or longer were aligned according to their non-U sequence. MURF2 pre-edited and fully edited sequence, along with the previously identified gMURF2, are shown above. Editing sites which that the canonical sequence are highlighted in yellow, while editing sites matching modifications made by alt-MURF2-gRNA2 are highlighted in blue. Green highlighting represents editing sites whose editing is are correctly edited by either gMURF2 or alt-MURF2-gRNA. Sequences were classified according to their complementarity to the canonical gRNA-MURF2 (Canon), alt-MURF2-gRNA2 (Alt), as hybrid junctions (*), or as undeterminable (?). (B) Quantification of junction classes shown in (A). Canon, canonical as specified by gMURF2; Alt, alternative as specified by alt-MURF2-gRNA2; Hybrid; containing 9 U's at ES447, but canonical sequences both 5' and 3' of this site.

Fig. S4. Overall changes in junction lengths. The percentage of normalized sequences (excluding pre-edited) with small (0-2) and long (11-13) junction lengths were calculated across (n=8) uninduced and (n=2) induced replicates for each knockdown. Error bars represent one standard deviation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ (Student's T-test).

Fig. S5. Sequence analysis of the most abundant junctions arising at ESS446 in Uninduced, TbRGG2, and GAP1 cell lines. (A) For each cell type, the renormalized counts of partially edited sequences were averaged (Avg ReNorm) across all uninduced cell lines used in this study (n=8) and induced knockdown cells (n=2 for each cell line). To evaluate gRNA usage, the most abundant 16 junctions comprised of 11 editing sites or longer were aligned according to their non-U sequence. MURF2 pre-edited and fully edited sequence, along with the gMURF2, alt-MURF2-gRNA1, and alt-MURF2-gRNA2 are shown above. Editing sites that match the canonical sequence are highlighted in yellow while editing sites matching modifications made by alt-MURF2-gRNA2 are highlighted in blue. Green highlighting represents editing sites that are correctly edited according to either gRNA. Sequence types (Type) were classified according to

complimentary to the canonical gRNA-MURF2 (Canon), alt-MURF2-gRNA2 (Alt), as hybrid junctions (*), or as indeterminable (?). Lower case u's are those inserted by editing, U indicates encoded uridines, and * indicates encoded uridines deleted by editing. (B) Quantification of junction classes shown in (A).